## REMARKS

The Office is authorized to charge the fee for a three month extension of time to Deposit Account No. 02-1818. Any fees that may be due in connection with the filing of this paper or with this application may be charged to Deposit Account No. 02-1818. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

A supplemental information disclosure statement is provided herewith.

Claims 1-7, 9, 11-16, 45, 48, 50-54, 56-59, 61-63, 65-78 are pending in this application. New claims 67-78 are added. Claims 1, 7, 16, 45, 48, 50-54, 57-59 and 63 are amended for clarity and to correct minor typographical and spelling errors. Amendments to claims 1, 53, 59 and 63 are also made to specify that the substrate sequence is a sequence in the target protein, whereby cleavage of the substrate sequence in the target protein inactivates an activity of the target protein. Basis for this amendment is found throughout the specification, for example, at paragraph [0050] and at paragraph [0125]. Amended claims 7, 53, 59 and 63 find basis in the specification, for example, at paragraph 0009, at paragraph 0063 and in Table 2. New claims 67-78 are added and find basis throughout the specification as filed, for example, at paragraphs [0107] and [0125]. Claims 60 and 64 are cancelled without prejudice. No new matter is added.

### I. THE REJECTION OF CLAIMS 1-7, 9, 11-16, 45-48, 50-54 AND 56-66 UNDER 35 U.S.C. §103

Claims 1-7, 9, 11-16, 45-48, 50-54 and 56-66 are rejected under 35 U.S.C. §103(a) as being unpatentable over Harris *et al.* I (*J. Biol. Chem.*, 273: 27364-27373 (1998)) or Harris *et al.* II (Harris *et al.* II (Current Opinion in Chemical Biology, 2:127-132 (1998)). The Examiner urges that "all of the elements of the claimed method are fully described by Harris *et al.* ( I)." The Examiner also states that claims 16, 45-46 and 53 are "obvious over the disclosure of Harris of the known iterative process of phage display method."

Applicant respectfully traverses this rejection as to each of Harris *et al.* I and Harris *et al.* II for the reasons of record and as further set forth herein. Each rejection is discussed in turn below.

**Relevant Law**

The relevant law was set forth in the previous response, mailed August 7, 2008, and is incorporated by reference herein. To establish *prima facie* obviousness under 35 U.S.C. §103, all the claim limitations must be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). This principle of U.S. law regarding obviousness

was not altered by the recent Supreme Court holding in KSR International Co. v. Teleflex Inc., 127 S.Ct. 1727, 82 USPQ2d 1385 (2007). In KSR, the Supreme Court stated that "Section 103 forbids issuance of a patent when 'the differences between the subject matter sought to be patented and the prior art are such the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains.'" KSR Int'l Co. v. Teleflex Inc., 127 S.Ct. 1727, 1734, 82 USPQ2d 1385, 1391 (2007).

The question of obviousness is resolved on the basis of underlying factual determinations including (1) the scope and content of the prior art, (2) any differences between the claimed subject matter and the prior art, (3) the level of skill in the art. Graham v. John Deere Co., 383 U.S. 1, 17-18, 148 USPQ 459, 467 (1966). See also KSR, 127 S.Ct. at 1734, 82 USPQ2d at 1391 ("While the sequence of these questions might be reordered in any particular case, the [Graham] factors continue to define the inquiry that controls.") The Court in Graham noted that evidence of secondary considerations, such as commercial success, long felt but unsolved needs, failure of others, etc., "might be utilized to give light to the circumstances surrounding the origin of the subject m*att*er sought to be patented." 383 U.S. at 18, 148 USPQ at 467. Furthermore, the Court in KSR took the opportunity to reiterate a second long-standing principle of U.S. law: that a holding of obviousness requires the fact finder (here, the Examiner), to make explicit the analysis supporting a rejection under 35 U.S.C. 103, stating that "rejections on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness. Id. at 1740-41, 82 USPQ2d at 1396 (citing In re Kahn, 441 F.3d 977, 988, 78 USPQ2d 1329, 1336 (Fed. Cir. 2006)).

While the KSR Court rejected a rigid application of the teaching, suggestion, or motivation ("TSM") test in an obviousness inquiry, the Court acknowledged the importance of identifying "a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does" in an obviousness determination. KSR, 127 S. Ct. at 1731. The court stated in dicta that, where there is a "market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to try **might** show that it was obvious under § 103."

In a post-KSR decision, PharmaStem Therapeutics. Inc. v. ViaCell. Inc., 491 F.3d 1342 (Fed. Cir. 2007), the Federal Circuit stated that:

> an invention would not be invalid for obviousness if the inventor would have been motivated to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful. Likewise, an invention would not be deemed obvious if all that was suggested was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.

Furthermore, KSR has not overruled See In re Papesch, (315 F.2d 381, 137 USPQ 43 (CCPA 1963)), In re Dillon, 919 F.2d 688, 16 USPQ2d 1897 (Fed. Cir. 1991), and In re Deuel (51 F.3d 1552, 1558-59, 34 USPQ2d 1210, 1215 (Fed. Cir. 1995)). "In cases involving new compounds, it remains necessary to identify some reason that would have led a chemist to modify a known compound in a particular manner to establish prima facie obviousness of a new claimed compound." *Takeda v. Alphapharm*, 492 F.3d 1350 (Fed. Cir. 2007).

The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); see, also, *In re Papesh*, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963). In addition, if the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims prima facie obvious. *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959).

As always, unexpected properties must always be considered in the determination of obviousness. A compound's structure and properties are inseparable so that unexpected properties are part of the subject m*att*er as a whole. *In re Papesch*, 315 F.2d 381, 391, 137 USPQ 43, 51 (CCPA 1963)

The disclosure of the applicant cannot be used to hunt through the prior art for the claimed elements and then combine them as claimed. In re Laskowski, 871 F.2d 115, 117, 10 USPQ2d 1397, 1398 (Fed. Cir. 1989). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" W.L. Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

It respectfully is submitted that, in this instance, the cited references singly, or in combination, doe not teach or suggest all of the limitations of the claimed method, nor teach or suggests the results achieved to identify proteases that inactivate an activity of a target protein involved in a disease or pathology, thereby identifying candidate therapeutics.

**The Claims**

The claims are directed to methods for identifying protease muteins as candidate therapeutics that cleave a substrate sequence in **a target protein that is involved with or causes a disease or pathology, thereby inactivating an activity of the target protein**. To practice the method, a **library of proteases and/or catalytically active portions thereof**, where each member of the library contains a mutation or mutations in a scaffold protease (*i.e.*, a wild-type protease) or a catalytically active portion thereof, is produced. The activity and/or specificity of members of the library in cleaving a substrate sequence in the target protein is assessed. Those members of the library that have increased cleavage activity and/or specificity for a substrate sequence are identified and selected. The targets are selected such that cleavage by a library member inactivates it and its inactivation renders the library member a candidate therapeutic for treatment of the disease or pathology. Thus, the identified modified protease(s) or a catalytically active portion thereof inactivates an activity of a target protein that is involved with or causes a disease or pathology, and, thus, is a candidate therapeutic for treating such disease or pathology. The independent claims recite particulars of the components (target protein and/or protease scaffold) of the method and/or particular steps of the method. For example, independent claim 1 recites:

> A method of producing and identifying a mammalian protease mutein that inactivates an activity of a target protein involved with a disease or pathology in a mammal, wherein:
>
> the target protein is selected from among a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis whose inactivation can ameliorate a disease or pathology;
>
> cleavage of a substrate sequence in the target protein inactivates an activity of the target protein; and
>
> the method comprises the steps of:
>
> (a) producing a library of protease muteins of a protease scaffold and/or catalytically active portions thereof, wherein:
>
> each different mutein protease in the library is a member of the library;
>
> each member of the library has N mutations relative to a wild-type mammalian protease scaffold or a catalytically active portion thereof; and
>
> N is a positive integer;

(b) contacting members of the library with the target protein or with a polypeptide comprising a substrate sequence that is present in the target protein;

(c) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the target protein or a substrate sequence in the target protein; and

(d) based on the measured activity and/or specificity, identifying those members of the library that have an increased cleavage activity and/or altered substrate specificity for cleaving the substrate sequence or the target protein, relative to the wild-type mammalian protease scaffold, thereby identifying a protease mutein or a catalytically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology, whereby the identified protease is a candidate therapeutic for treatment of the disease or pathology.

Independent claim 53 further recites additional steps of the method and also recites particular scaffold proteases used in the method. Independent claim 59 recites that the protease is a human protease and further recites the particular scaffold proteases used in the method. Independent claim 63 recites specific target proteins and also recites that the proteases scaffold is a human protease and recites specific protease scaffolds used in the method. All claimed methods employ **a library of protease muteins** (not a library of substrates as stated by the Examiner throughout with respect to the Harris *et al.* I reference) based on a particular scaffold that include mutations. Identified proteases are those that have increased cleavage activity and/or substrate specificity for the target protein compared to the wildtype scaffold protease not including the mutations. Dependent claims recite particular proteases and/or targets as well as other particulars regarding the methods and libraries.

**Analysis**

***1. Harris et al. I (J. Biol. Chem., 273: 27364-27373 (1998)***

***Teachings of Harris et al. I***

As discussed in the previous response, Harris *et al.* I is directed to a method of **determining** the substrate specificity of purified wild-type rat granzyme B. Harris *et al.* I teaches two different methods for identifying **substrate residues** that are recognized by wild-type granzyme B. In both methods of Harris *et al.*, wild-type recombinant rat granzyme B is the protease whose substrate specificity is assessed. In the first method, substrate specificity of granzyme B is assessed by assaying the activity of the purified rat wild-type granzyme B for a **library of substrates** to **identify substrates that granzyme B cleaves**. Thus in this method, substrates that are cleaved by granzyme B are identified (not modified proteases that cleave a particular substrate).

Specifically, in the first method, Harris *et al.* assesses granzyme B cleavage of different tetrapeptide substrates by identifying residues in a granzyme B **substrate** that represent the P1-P4 amino acids cleaved by granzyme B. Harris *et al.* teaches identification of the optimal substrate residue in the P1 position of the substrate, by testing cleavage of the tetrapeptide substrates "which contained various amino acids at the P1 position" (see *e.g.*, page 27367, first full paragraph). Harris *et al.* teaches that Asp is the optimal residue at the P1 position of the substrate. After identification of the P1 residue of the optimal granzyme B substrate, Harris *et al.* teaches identification of the "Extended (P4-P2) Substrate Specificity" using a "positional scanning combinatorial substrate library (PS-SCL)." The PS-SCL is composed of three sub-libraries containing a total of 8000 tetrapeptide substrate members. The combinatorial substrate phage library is a library of tetrapeptide (4 amino acid) substrates having the general structure of Ac-$Xaa_{P4}$-$Xaa_{P3}$-$Xaa_{P2}$-Asp-AMC. Each member of the library represents a potential substrate for granzyme B, and each substrate variously differs in the P4-P2 positions, while the P1 position of the substrate remains constant. The library provides 8000 tetrapeptide substrates and each was tested to determine if granzyme B cleaves the substrate. The use of the AMC fluorogenic moiety permits identification of substrates that are cleaved by granzyme B. Harris *et al.* I teaches that "analysis of the three libraries affords a complete understanding of enzyme preference for amino acids at P4, P3 and P2" positions of the substrate (see e.g. at page 27367, beginning of second column). Hence, this method relies on varying the tetrapeptide substrate, and assessing the cleavage activity of wild-type rat granyme B. Analysis of cleavage of the tetrapeptide substrates by wild-type granzyme B is used to identify an optimal substrate of granzyme B. Using the combinatiorial substrate library, Harris *et al.* I teaches that the optimal P4 to P2' granzyme B cleavage site is (Ile>Val)(Glu>Gln= Met)Xaa-Asp/ Xaa-Gly, for example, the substrate sequence IEPD/.

Based on the elucidation of this substrate specificity of granzyme B, Harris *et al.* also teaches that potential *in vivo* targets of granzyme B can be identified. For example, Harris *et al.* teaches that, based on the substrate specificity of granzyme B, certain caspases (caspases 3 and 7) , based on their sequences, are more likely substrates then other caspases.

Hence, as taught by Harris *et al.*, the above method is used to assess the specificity of a wild-type granzyme B polypeptide for a substrate from among a library of **8000 different substrates,** which information was used to identify a potential *in vivo* substrate of rat granzyme B. Harris *et al.* then is directed to **substrate libraries** used to identify substrates recognized by wild-type granzyme B. As discussed below, and is apparent, this method is completely different from

the instantly claimed method, which identifies modified proteases from among a library of proteases, that can inactivate a target involved in a pathology or disease on cleavage of a particular substrate.

In the second method, an homology model is used to identify residues in a model substrate that could interact with residues in rat wild-type granzyme B. Using this approach, one residue in granzyme B (Arg192) is identified as an important residue for substrate specificity. To confirm its importance, cleavage by two granzyme B polypeptides having mutations at this position, R192A and R192Q were tested. The homology model method used in this second method, involves an analysis of the three-dimensional model of the enzyme-substrate complex to identify residues that are structural determinants i.e. residues in granzyme B that potentially interact with the substrate (see e.g. Figure 4 of the Harris *et al.* I reference). The method actually models Rat mast cell protease, which exhibits 48% sequence identify to granzyme B. This method involves no actual assessment of cleavage activity and/or activity of the granzyme B for the substrate. Rather, potential substrate residues and enzyme residues are identified that could be potential sites of interaction based on modeling alone. By using this method, Harris *et al.* teaches only the identification of Arg192 in granzyme B as a potential specificity determinant. Harris *et al.* teaches that Arg192 is an important specificity determinant because mutation of Arg192 to alanine or glutamate results in an enzyme with decreased activity for the tetrapeptide substrate IEPD compared to the wild-type enzyme. This second method in Harris *et al.* I bears no resemblance to the instantly claimed methods.

***Differences between the teachings of Harris et al. I and the instant claims***

Accordingly, as set forth in the previous response, Harris *et al.* I is of no relevance to the instant claims, since it fails to teach or suggest **any** element of the instantly claimed methods. The instant claims are directed to a method of identifying a mutein protease that inactivates an activity of a target protein involved in a disease or pathology for use as a candidate therapeutic. The method includes a) producing a **library of proteases muteins (not a library of substrates)** each having N mutations relative to a scaffold protease; b) contacting members of the library with a target protein or protein containing a substrate sequence from the target protein; c) measuring a cleavage activity and/or specificity for a substrate sequence in the target protein; and d) identifying those members of the library that have an increased cleavage activity and/or altered substrate specificity relative to the wild-type protease scaffold. The target protein is selected so that it inactivation can ameliorate the symptoms of

a disease or pathology. Harris *et al.* I does not teach or suggest any of these steps or aspects of the instantly claimed method.

*a. Harris et al. does not teach or suggest anymethod that employs any protease library*

Harris *et al.* I is directed to the elucidation of the substrate specificity of **wild-type granzyme B**. The point of Harris *et al.* I is to identify residues in substrates that are involved in protease substrate specificity of a wild-type granzyme B. Harris *et al.* employs a substrate library that includes 8000 tetrapeptide substrates that are potential substrates of granzyme B. Thus, contrary to the Examiner's comments in the response (see rebuttal below), Harris *et al.* I does not teach a granzyme B protease library. No protease libraries are taught or suggested in Harris *et al.* I. Harris *et al.* also identifies one residue in wild-type granzyme B protease that is important for substrate specificity. The two modified granzyme B proteins produced contain a mutation at residue 192 to confirm its importance in substrate specicity. The modified granzyme B proteins are not designed or selected to inactivate or even cleave a target; they are produced to show that modification of this position impairs cleavage or specificity. Hence, Harris *et al.* cannot teach or suggest the instant claims, which require a step of producing a library of protease muteins.

*b. Harris et al. does not teach or suggest a step of contacting **members of a protease library** with **a substrate** or **a target.***

Harris *et al.* assesses the activity of wild-type granzyme B against a library of different substrates. In contrast, the instant claims require contacting members of a **protease library** against a substrate or target.

*c. Harris et al. does not teach or suggest a method including a step of measuring the cleavage activity and/or substrate specificity of a substrate sequence in a target protein*

The instant claims include a step of measuring the cleavage activity and/or substrate specificity of members of the library for a substrate sequence in a target protein. While Harris *et al.* does not even teach or suggest a protease library, Harris *et al.* I also does not teach or suggest a step measuring the cleavage activity of **a substrate sequence in a target protein <u>as claimed</u> that is involved in a disease or pathology**. Harris *et al.* does not teach measuring cleavage activity of any granzyme B mutein nor even wild-type granzyme B for a caspase. Harris *et al.* identifies particular caspases as potential substrates for wild-type granzyme B based upon the presence of the identified granzyme B canonical substrate sequence in the particular caspases.

The tetrapeptide substrate sequence, IEPD, which was used to assess cleavage of the

two granzyme B muteins R192A and R192E, is not a substrate sequence in a target protein involved in a disease or pathology. It is the identified optimal substrate sequence for granzyme B. The IEPD substrate sequence, however, is not contained in any of the caspase target substrate sequences (see e.g. Figure 5D). Thus, there is no teaching or suggestion in Harris *et al.* I of a method including a step of measuring the cleavage activity and/or substrate specificity of protease muteins for a substrate sequence in a target protein involved in a disease or pathology.

d. *Harris et al. I does not teach or suggest a method that includes a step of identifying a protease mutein that inactivates an activity of a target protein for use as a candidate therapeutic*

Notwithstanding the above, Harris *et al.* I also does not teach or suggest a method as claimed of identifying protease muteins that inactivate an activity of a target protein by identifying those that have increased cleavage activity and/or altered substrate specificity for **a substrate sequence in a target protein involved in a disease or pathology**, whereby cleavage of the substrate sequence inactivates an activity of the target protein. Hence, there is no teaching or suggestion in Harris *et al.* I of identifying a protease mutein as a candidate therapeutic. There is no mention at all or even a suggestion of using proteases as candidate therapeutics. The instant claims are directed to a method the includes identifying protease muteins that **inactivate** an activity of a target protein involved in a disease or pathology. Harris *et al.* does not teach or suggest any method of identifying a protease mutein that inactivates an activity of a target protein, in particular a method involving identifying mutein proteases that have increased cleavage activity and/or substrate specificity for a substrate sequence in the target protein. In fact, the substrates of wild-type granzyme B taught in Harris *et al.* I are caspases, which require cleavage by granzyme B for **activation**. In contrast, the instant application teaches a method to modify granzyme B to inactivate an activity of a target protein such as caspase (as exemplified in the instant application, see e.g. Example 11 regarding identification of a granzyme B protease mutein to inhibit apoptosis by inactivation of caspase-3). Hence, Harris *et al.* I does not teach or suggest a method of identifying candidate therapeutics by identifying mutein proteases, including mutein granzyme B proteases, that have increased cleavage activity and/or altered specificity for a target protein involved in a disease or pathology as instantly claimed.

***Conclusion***

Thus, Harris *et al.* I is deficient in failing to teach or suggest any elements of the instantly claimed methods. Harris *et al.* I fails to teach or suggest preparing a library of

**mutein proteases**; assessing cleavage activity and/or substrate specificity for a particular substrate sequence in a **target protein involved in a disease or pathology** where cleavage of the substrate sequence in the target protein **inactivates** an activity of the target protein; and identifying any in the library that has increased cleavage activity and/or substrate specificity for the substrate sequence in the target protein compared to the scaffold protease not containing the N mutations for the target, thereby identifying a candidate therapeutic. Thus, Harris *et al.* is of no relevance. Thus, it respectfully is submitted that the Examiner has failed to set forth a prima facie case of obviousness.

***Rebuttal to the Examiner's comments***

*a. The Substrate Libraries of Harris et al. I are not the same as the Protease Libraries as instantly claimed*

While it appears that the Examiner recognizes that Harris *et al.* I teaches a method directed to substrate libraries, it appears that the Examiner believes that these substrate libraries are the same as the protease (e.g. mutein granzyme B protease) libraries as instantly claimed. For example, the Examiner states that Harris *et al.* I teaches, throughout the article, a method of providing a mutein library of granzyme B with substrate specificity and teaches the construction of Granzyme B variants, especially in the method of creation of P3, P1', P2' his-tagged **substrate** phage display library. In particular, the Examiner cites e.g. page 27365, col. 2, which recites:

> The vector contains the following amino acid sequence.... residues 198-406 of pIII coat protein of MI3 bacteriophage: AESVQPLGPG.HHHHHHHGHAGIXPDXXAGPGGG...The degenerate oligonucleotides synthesized to create the library consisted of the following sequence (where N indicates equimolar concentrations of G, C, A, and T; S indicates equimolar concentrations of G and C): CAT GGG CAT GCA GGA ATT NNS CCA GAC NNS NNS GCA GGG CCC GGA GGC GGT CCA TTC GTT.... The library of <u>substrate phage</u> has 32,768 possible DNA sequences that translates into 8000 possible protein sequences. Phage particles expressing the engineered pIII substrate fusion protein were prepared as described previously (26)...Phage particles... were added to the washed Ni(II) resin and allowed to bind with gentle agitation for 3 h. The Ni(II) resin was then washed... to remove unbound phage... the cleaved phage were separated from the resin...The cleaved phage were amplified.... to form recombinant phage which were then usual for the **next round of cleavage selection.** After four rounds of cleavage selection.... Twenty individual colonies were selected and grown.... and plasmid DNA was isolated and sequenced in the region of the cleavage site. [Emphasis provided by the Examiner in bold; Emphasis provided by Applicant in underline]

And further cites at page 7 of the Office Action from page 27367 of the Harris *et al.* I reference:

> A positional scanning combinatorial substrate library (PSSCL) was used to elucidate the specificity of purified, recombinant, rat granzyme B. This library, of the general structure Ac-Xaap4-Xaam-Xaap2- Asp-AMC, has been previously used to identify the amino acid preferences of the caspases and human granzyme B purified from cultured natural killer leukemia YT cells (27). The PS-SCL is composed of three libraries, each of which consist of 20 sublibraries, for a total of 4000 compounds. In each sublibrary, one position (P4, P3, or P2) contains a defined amino acid and the other two positions contain an equimolar mixture of amino acids (two unnatural amino acids, D-alanine (D-A) and nor leucine (n), are included; eysteine and methionine are excluded). Thus analysis of the three libraries affords a complete understanding of **enzyme preferences for amino acids at P4, P3, and P2.** This approach has been previously validated as providing an accurate measure of protease specificity using caspase-1....The PS-SCL indicates that granzyme B can accept a broad range of amino acids in the P2 position, although proline is the preferred amino acid.....The positional scanning synthetic combinatorial library suggests that granzyme B exhibits unique and extended substrate specificity.... [Emphasis provided by the Examiner in bold; Emphasis provided by Applicant in underline].

It respectfully is submitted that the teachings of Harris *et al.* I cited by the Examiner above are directed to a substrate phage display library; this is **NOT** a library of Granzyme B variants as stated by the Examiner. The passages cited above render it clear that the specificity of a purified, recombinant wild-type rat granzyme B, and not a library of granzyme B variants, is being tested for specificity and enzyme preference for a **substrate library**, whereby a substrate is an amino acid sequence recognized by and cleaved by the wildtype purified granzyme B protein (see underlined portions of passages underlined by Applicant for Emphasis). In contrast, **the instant claims are directed to a method of screening a protease library; not a substrate library.** The method as claimed includes no step of using a substrate library.

*b) Harris et al. does not teach or suggest producing a library of mutein proteases as required in the method as instantly claimed*

The Examiner further states that Harris *et al.* provides a mutein library of granzyme B, see e.g. bottom of page 5-page 6 of the Office Action, citing page 27365, col. 1, first incomplete paragraph:

> ...Two combinatorial methods to extend the definition of the substrate specificity of granzyme B to six subsites from P4 to P2. Individual amino acids responsible for determining **stringent substrate specificity** of granzyme B were identified through the construction of a three-dimensional model of granzyme B complexed to substrate. **Variant granzyme B with altered P1 and P3 substrate recognition properties were created** (reads on the claimed

> library) **to define the molecular determinants of specificity** (reads on the claimed altered specificity). The elucidated substrate specificity was shown to be relevant within a macromolecular context by locating cleavage sites in defined molecular targets. [Emphasis added by the Examiner in bold; parentheticals added by the Examiner].

In particular, the Examiners comments in parentheticals in the above passage states that the variant granzyme B that were generated read on a method that employs a library of proteases. **It respectfully is submitted that Harris *et al.* I did not create a library of granzyme B mutants.** There is no disclosure, teaching or suggestion of a library of granzyme B mutants in Harris *et al.* I. Rather, based on the homology modeling, Harris *et al.* teaches identification of amino acid position Arginine 192 as a potential structural determinant of specificity of granzyme B. Harris *et al.* teaches the generation and testing of **two** granzyme B mutants, R192E and R192A, and teaches that such mutants exhibit reduced hydrolysis of the optimal tetrapeptide substrate Ac-IEPD-AMC and non-optimal tetrapeptide substrate Ac-IKPD-AMC compared to the wild-type enzyme. Hence, Harris *et al.* does not teach generation of a library of granzyme B mutants as required by the instant claims.

Accordingly, it is respectfully submitted that all claims include a step of producing a library of mutein proteases, which is not taught or suggested by Harris *et al.* Therefore, the Examiner's further statements that claims 16, 45-46 and 53 are obvious over the disclosure of Harris and the known iterative process of phage display is also inapt, since Harris *et al.* does not teach or suggest phage display of proteases. For example, in reaffirming the rejection of claims 16, 45-46 and 54, the Examiner cites the Experimental Procedures section of Harris *et al.* I (e.g. page 27365 sic, col. 2; note this should be page 27366, col.1), and states that Harris *et al.* I " describes the well known phage process of iterative selection to obtain, e.g., enzymes with increased property e.g., specificity (e.g. the known phage biopanning)." As discussed above, it respectfully is submitted that the phage library taught by Harris *et al.* I is a **substrate** phage display library containing tetrapeptide substrates. It is not a protease phage display library, and contrary to the Examiner's comments, was not used to identify enzymes with increased property. For example, the Examiner's attention is drawn to the Title of the recited Section in the Experimental Procedures, which is "His-tagged **Substrate** Phage Cleavage Assay." Further, a portion of the text not quoted by the Examiner states that "Recombinant granzyme B was added [to the phage particles] to a final concentration of 10 nM." Hence, it is clear that the phage library itself is a library of substrates coupled to a nickel residue by the existence of a His-tag. Wild-type granzyme B was added to the phage. Cleavage of the His-tagged substrate by granzyme B

resulted in release of the phage particle from the nickel resin and separation from the resin for subsequent identification.

*c) Harris et al. does not teach a method that uses a scaffold protease as claimed, since Harris et al. does not teach or suggest a method including a step of producing a library of mutein proteases, each mutein having N mutations in a scaffold protease*

The Examiner states that Harris *et al.* I teaches use of Rat mast cell protease-2 (RMCP-2) in the modeling, indicated by the Examiner as being a "scaffold, as claimed" (see e.g. at page 7 of the Response, top of the page, quoting the reference "...Rat mast cell protease-2 (RMCP-2 (28) was used as the **framework** (scaffold, as claimed) to model rat granzyme B since they share 49% sequence identity." It respectfully is submitted that a method using homology-built three dimensional model is not relevant to the instant claims, which are directed to a method of identifying protease muteins that inactivate an activity of a target protein involved in a disease or pathology by measuring cleavage and/or activity. The use of RMCP-2 as described in Harris *et al.* I was used as a "framework" insofar as it was used to model the interaction of granzyme B with a substrate due to its high degree of homology to granzyme B (48%) and because the 3-D structure of RMCP-2 is known. In contrast, as claimed, a scaffold is a starting unmodified protease (e.g. wild-type protease) for which mutations are introduced, thereby producing a library of protease muteins each containing N mutations relative to the scaffold protease. Harris *et al.* does not teach or suggest producing a library of protease muteins from a starting scaffold protease, nor a method of using such a library to identify protease muteins that have altered cleavage activity and/or substrate specificity for a substrate sequence in a target protein involved in a disease or pathology.

*d) The Examiner's comment that Harris et al. I teaches caspase as a target protein involved in a disease or pathology is inapt, since Harris et al. does not teach or suggest a method of modifying a protease to inactivate a caspase to treat a disease or pathology. In Harris et al. I, Caspase is merely identified as a natural substrate of wild-type rat granzyme B.*

The Examiner states that identification of "caspases" as substrates for granzyme B "reads on target protein involved with a pathology." For example, the Examiner cites page 27372 up to and 27373:

> {T]here is a functional relationship between the preferential substrate sequence of granzyme B and the activation site of members of the caspases (Fig. 5D). Indeed, studies have shown that granzyme B cleaves and activates several **caspases involved in apoptosis** (reads on target protein involved with a pathology). Our data on the substrate specificity of granzyme B suggest that caspase 3 and caspasc 7 are preferentially activated during apoptosis. Knowledge of the extended substrate specificity of granzyme B allows for the proposal of additional targets of granzyme B during apoptosis. The substrate specificity of caspase 6 matches that of granzyme B (27), suggesting that both

> enzymes cleave the same substrates. Several proteins known to be cleaved during apoptosis, such as nuclear lamin A....
> The identification of their specificity will further expand our knowledge of the role that granzymes play in **cytotoxic, lymphocyte-mediated cell death.** (Emphasis added).

It respectfully is submitted that the cited passage is irrelevant to the instant claims. As set forth in the passage above, it is well known in the art that caspase is an in vivo substrate of **wild-type granzyme B** and is required for the activation of some caspases in vivo. The cited passage merely teaches that elucidation of the substrate specificity of wild-type granzyme B will aid in the understanding and knowledge of the substrates of granzyme B and in vivo role of **wild-type granzyme B** in particular activities, such as lymphocyte-mediated cell death. The fact that Harris *et al.* states what is known in the art, i.e. that some caspases are substrates for granzyme B, is not dispositive of a prima facie case of obviousness, since Harris *et al.* I lacks any teaching or suggestion of any of the elements of the method of identifying mutein proteases as claimed. The instant claims are directed to a method of identification of **mutein proteases**, such as mutein granzyme B proteases, that inactivate a target protein involved in a disease or disorder by virtue of having an altered cleavage activity and/or substrate specificity for a target protein involved in a disease or disorder compared to the wild-type protease. Hence, the instant claims are directed to a method of identifying mutein proteases as candidate therapeutics for use in inactivating the activity of a target protein involved in a disease or disorder.

Importantly, there is no teaching or suggestion in Harris *et al.* I of a method of modifying any protease, including granzyme B, to have altered cleavage activity and/or substrate specificity for a target protein involved in a disease or disorder (e.g. caspase). In fact, for the two mutants of granzyme B that were made, they were made for the purpose of confirming that Arg192 is a determinant of specificity of granzyme B. The mutations were not made in order to identify a mutein granzyme B proteases that inactivates an activity of a caspase as instantly claimed. The substrate tested for cleavage by the mutants was an IEPD tetrapeptide substrate, identified as the optimal substrate of granzyme B. There is no teaching or suggestion in Harris *et al.* I that the IEPD substrate is a substrate sequence in a target protein involved in a disease or pathology. In fact, among the elucidated potential caspase substrates of granzyme B identified in Harris *et al.* (set forth in Figure 5D), **NONE** contain the tetrapeptide substrate IEPD. There is no teaching or suggestion of assessing or measuring the cleavage activity and/or substrate specificity of a protease, e.g. mutant granzyme B proteases, towards substrates involved in a disease or pathology to identify a mutein protease for use as a candidate therapeutic via inactivation of an activity of the

target protein.

Furthermore, Harris *et al.* I teaches that granzyme B "mediates proteolytic **activation** of the apoptotic class of caspases" (see e.g. at page 27371, first paragraph of the Discussion). The instant claims are directed to identification of mutein proteases that have altered cleavage activity and/or substrate specificity for a substrate sequence in a target protein, whereby cleavage of the substrate sequence inactivates an activity of the target protein. There is no teaching or suggestion in Harris *et al.* of a method of identifying mutein granzyme B, nor any mutein granzyme B, that inactivates a target protein involved in a disease or pathology.

***2. Harris et al. II (Current Opinion in Chemical Biology, 2:127-132 (1998))***

***Teachings of Harris et al. II***

Harris *et al.* II is a review article directed to methods for identifying determinants of protease specificity and for modifying specificity as a means to "understand the factors involved in protease specificity." Harris *et al.* II teaches that several methods have been employed to make changes in enzyme specificity in order to understand protein design principles, including rational and random (irrational) approaches to protein design. The first method, designated rational redesign specificity, requires a detailed understanding of the catalytic mechanism and sequence determinants for a particular protease as basis for predictably altering specificity. Harris *et al.* II also teaches complete random mutagenesis and states that this requires large libraries of mutants to identify a desired function. Another method, employs comparative analysis using homologous proteins that differ in substrate specificity. In another method, Harris *et al.* II describes the use of alanine scanning to identify residues involved in activity, followed by replacement of identified residues with all 19 amino acids to produce modified proteins with altered activity. In a final method, Harris *et al.* teaches using directed evolution, such as DNA shuffling methods, to generate enzymes with modified activities.

Harris *et al.* II teaches that such protein engineering aids in the elucidation of substrate specificity of a protease, i.e. the preference that an enzyme has for one substrate over a competing substrate. For example, Harris *et al.* reviews a method of protein design of Shokat and coworkers to modify a kinase to identify the direct substrates of the kinase. Harris *et al.* II also reviews studies where redesign of a protease was based on comparative analysis of homologous enzymes. For example, a study of Ballinger *et al.*, compared the specificity determinants of three enzymes (bacterial subtilisin BPN', kex2 and furin) to identify residues to modify one of the enzymes (subtilisin BPN') to have the substrate

specificity of one of the other enzymes (kex-2). A similar study using comparative analysis from Dean and coworkers is reviewed. Harris *et al.* II also teaches that protein engineering can be used to alter the substrate specificity of a protein towards a natural substrate to alter activation of substrates. For example, Harris *et al.* II provides a review of Tsiang *et al.* (Biochemistry (1996) 3:16449), whereby random mutagenesis was used to identify a mutant thrombin that has increased specificity for one natural substrate (Protein C) compared to another natural substrate. Naturally, activation of protein C and fibrinogen is mediated by cleavage by thrombin. Hence, according to Harris *et al.* II, the study identifies a mutant protease with preferential protein C activation over fibrinogen cleavage. Other uses of redesign taught by Harris *et al.* II based on a review of the literature include identification of protease mutants that are more sensitive to chemotherapeutic drugs; identification of protease mutants to look at resistance profile of an antibiotic (cefotaximine derivatives) to obtain antibiotics less prone to resistance; and modification of an enzyme to improve its fucosidase activity compared to its galactosidase activity.

***Differences between the teachings of Harris et al. II and the instant claims***

The instant claims are directed to a method of identifying a protease mutein that **inactivates** an activity of a target protein involved in a disease or pathology. The claimed methods, which are discussed above, include a step of measuring the cleavage activity and/or substrate specificity for a substrate sequence in a target protein involved in a disease or pathology, whereby cleavage of the substrate sequence in the target protein inactivates an activity of the target protein. The claims recite particular categories of target proteins and specific target proteins involved in a disease or pathology. The target protein is selected so that its cleavage inactivates it **and its inactivation** can ameliorate the symptoms of a disease or pathology. Thus, the instant claims are directed to identifying protease muteins that are candidate therapeutics, whereby inactivation of an activity of the recited target protein involved in a disease or pathology is a candidate treatment for the disease or pathology.

There is no teaching or suggestion in Harris *et al.* II of any method for identifying protease muteins that are candidate therapeutics and certainly no method in which a library of protease muteins is prepared and members contacted with a substrate sequence from a target or the target to identify those that cleave and inactivate the target to thereby selected modified proteases that are candidate therapeutics.

As discussed above, Harris *et al.* II is a review of the state of the art regarding "current uses of protein design," and summarizes "the uses of protein engineering in

elucidating substrate specificity determinants." Harris *et al.* II does not teach or suggest any method of identifying a mutein protease that has altered substrate specificity and/or cleavage activity for a substrate sequence in a target protein, whereby cleavage of the target protein **inactivates** an activity of the target protein. Hence, among the varied references reviewed by Harris *et al.*, none teaches or suggests a method for identification of a protease mutein that inactivates a target protein involved in a disease or pathology and whose inactivation ameliorates the symptoms of a disease or pathology, so that the identified protease mutein candidate therapeutic for treatment of the disease or pathology. There is no teaching or suggestion that a protease could be used as a candidate therapeutic by inactivating the activity of a target protein, nor a method for identifying such a protease nor a method that includes the steps of the instantly claimed method.

Notwithstanding the above, Harris *et al.* II does not teach or suggest a method of identifying a protease mutein that has altered specificity and or increased cleavage activity for a substrate sequence in a target protein for any of the recited target proteins involved in a disease or pathology as claimed. There is no teaching or suggestion in Harris *et al.* II of any method of identifying a protease that has increased cleavage activity and or/altered specificity for a substrate sequence in a target protein that is a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis (see e.g. independent claim 1 and 59 and claims dependent thereon), where inactivation of the target protein ameliorates the symptoms of a disease or pathology, nor any of the specific recited target proteins in independent claim 63 and claims dependent thereon; see also dependent claims 12, 62 and 66). There is no teaching or suggestion of a protease mutein that has altered cleavage activity and/or specificity for any such target proteins as claimed, whereby cleavage of the substrate sequences results in inactivation of an activity of the recited target proteins.

Further Harris *et al.* also does not teach or suggest many of the particulars of dependent claims. For example, Harris *et al.* II fails to teach or suggest an iterative screening method where, upon identifying a first protease mutein and a second protease mutein, the mutations are combined to generate a third protease mutein (see e.g. claim 53 and claims dependent thereon, and further dependent claim 16).

***Conclusion***

Thus, Harris *et al.* II is deficient in failing to teach or suggest the instantly claimed methods. Harris *et al.* II fails to teach or suggest any method of identifying a candidate

therapeutic that inactivates a target protein involved in a disease or pathology such that its inactivation results in amelioration of the symptoms of a disease or pathology. In particular, Harris *et al.* II does not teach any method that includes producing a library of protease muteins containing mutations in a scaffold protease, contacting the members of the library with the target protein or a protein comprising a substrate sequence in the target protein, measuring the activity and/or substrate specificity for a substrate sequence in a particular recited target protein involved in a disease or pathology, whereby cleavage of the substrate sequence inactivates an activity of the target protein, and identifying therefrom those members of the library that have an increased cleavage activity and/or substrate specificity for the susbstrate sequence relative to the wild-type scaffold to thereby identify candidate therapeutics for treating the disease or pathology. There is no teaching or suggestion in Harris *et al.* II of any methods that employ the categories of target proteins or specific target proteins as claimed. Accordingly, the Examiner has failed to set forth a prima facie case of obviousness.

***Rebuttal to the Examiner's comments***

In the previous response, Applicant provided arguments that none of the methods in Harris *et al.* II include a method as claimed for generating a mutein protease that has specificity for a particular target protein involved in a pathology. Applicant stated that there is no teaching or suggestion in Harris *et al.* II of modifying a protease to have increased cleavage activity and/or specificity for a substrate sequence **in a target protein involved in a pathology** nor any methods of doing so. In the present Office Action, the Examiner finds the arguments unpersuasive and draws the Applicant's attention to the paragraph bridging pages 129-130:

> A similar **therapeutic objective** in redesigning pre-existing enzymatic substrate specificity is the creation of `designer enzymes' to enhance the activity of small molecule drugs... A combination of random mutagenesis, genetic selection and drug-sensitivity screens was used to generate herpes simplex virus type I thymidine kinase (HSVI TK) variants that demonstrate substrate specificity towards the chain-terminating nucleoside analog pro-drugs ganciclovir and acyclovir. **The preferential phosphorylation of these pro-drugs would increase their chemotherapeutic value in treating particular cancers by enhancing the incorporation of the drug into DNA.** Based on the sequence conservation among the thymidine kinases and previous mutagenesis studies, six amino acids were targeted for saturation mutagenesis. These sites were changed to all other possible amino acids and the resulting **library of randomized HSV-I TKs was sequentially selected for thymidine kinase activity and screened for sensitivity in ganciclovir and acyclovir. A variant with**

> **four amino acid substitutions in the kinase active site resulted from the screen that was 43 times more sensitive to ganciclovir and 20 times more sensitive to acyclovir. This approach could provide a potentially useful tool for gene therapy applications.** (Emphasis added by Examiner).

It respectfully is submitted that the recited portion of Harris *et al.* is directed to the identification of a protease mutein that has increased specificity for a **small molecule drug**; cleavage of the prodrug activates the drug. Thus, the recited portion is related to a method for increasing the activity of a small molecule that is a prodrug. The recited portion is not directed to a method of identifying protease muteins from among members of a protease library that have altered substrate specificity and/or cleavage activity for a substrate sequence in **target protein involved in a disease or pathology**. The recited portion of Harris *et al.* II does not teach or suggest a method of identifying protease muteins for use themselves as candidate therapeutics for the treatment of a disease or pathology. There is no teaching or suggestion that a protease that serves to cleave and inactivate a target protein involved in a disease or pathology could be used to treat the pathology, nor any method of identifying such proteases.

## II. THE REJECTION OF CLAIMS 1-7, 9, 11-16, 45-48, 50-54 and 56-66 UNDER 35 U.S.C. §103

Claims 1, 2-7, 9, 11-16, 45-48, 50-54 and 56-66 are rejected under 35 U.S.C. §103(a) as being unpatentable over Kolterman *et al.(* (US 2004/0072276) in view of Waugh *et al.* (Nature Structure Biology) for the reasons of record in the last Office Action mailed February 07, 2008. Specifically, the Examiner alleges that Kolterman *et al.* teaches a method for generating sequence-specific proteases, and Waugh *et al.* teaches granzyme B. The Examiner concludes that it would have been obvious to one of ordinary skill to have used granzyme as the enzyme as taught by Waugh in the method of Kolterman, and that one of ordinary skill in the art would have been motivated to do so because of the advantage taught by Waugh that granzyme leads to rapid cell death of a tumor cell. This rejection is respectfully is traversed.

Responsive to the Examiner's statement that "applicants cannot attack the references individually when the rejection is based on the combination of references," Applicant respectfully submits that the references were not attacked individually. As discussed in the first obviousness rejection under the Section Header Relevant Law, a finding of obviousness requires that all elements of a claim are taught or suggested by the cited references, either alone or in combination. Thus, in the last response, the teachings of each reference were

discussed individually under the section "Differences Between the Claims and Teaching of the Cited References" to identify the differences in each reference compared to the elements of the method as claimed. Following that discussion, Applicant's provided an "Analysis" of the combination of references under the section entitled "The combination of teachings of the cited references does not result in the instantly claimed methods" (see page 54 of the Response mailed August 07, 2008). Accordingly, Applicant unequivocally addressed the combination of references.

In this Response, in the discussion below, each of the cited references is discussed,. Following the discussion of each reference the combined teachings and the deficiencies thereof are discussed, including rebuttal to the Examiner's comments regarding the references and Applicant's previous arguments. Applicant is not attacking each reference separately; but to assess the deficiencies of the combination of teachings, each reference is considered.

**Relevant Law and Rejected Claims**

The relevant law and rejected claims are discussed above in the first obviousness rejection.

**Summary of Arguments Below**

Besides stating that Applicant did not address the combination of references, the Examiner has provided no reason for why the combination of references supports a *prima facie* case of obviousness. As stated by the Examiner, the only basis for citing Waugh *et al.* as a reference is because of its teaching of granzyme B, since Kolterman *et al.* does not teach or suggest granzyme B. Applicant does not refute that Waugh *et al.* teaches the structure of granzyme B and the specificity determinants of wild-type granzyme B. As discussed in the last response, the teachings of Waugh *et al.* when combined with Kolterman *et al.*, however, does not teach or suggest the method as claimed. Kolterman *et al.* is deficient in teaching or suggesting the steps of the method as claimed, and Waugh *et al.* does not cure this deficiency by teaching the missing steps. In the instant Office Action, the Examiner repeats Applicant's arguments from the Response mailed August 07, 2008 rebutting a *prima facie* case of obviousness (see e.g. Page 14-17 of the instant Office Action), but fails to provide any reason why Applicant's arguments regarding the differences between the teachings of Kolterman *et al.* and Waugh *et al.* and the instant claims are not persuasive. Instead, the Examiner just state that "the combined teachings of Kolterman and Waugh will lead one having ordinary skill in the art to the claimed method."

It respectfully is submitted that the combination of teachings of Kolterman *et al.* and

Waugh *et al.* does not teach the claimed method. As quoted by the Examiner in the Office Action at page 18:

> When considering obviousness of a combination of known elements, the operative question is thus 'whether the improvement is more than the predictable use of prior art elements according to their established function.' KSR International Co. v. Teleflex Inc., 550 USPQ2d 1385 (2007).

Accordingly, as recognized by the Examiner, to support a *prima facie* case of obviousness over a combination of references, the method as claimed must at least be a combination of "prior art elements" taught or suggested by the references. As discussed in the previous response, neither Kolterman *et al.* or Waugh *et al.*, singly nor in any combination, teaches or suggest sall elements of the method as set forth in the claims. For example:

1) The library of Kolterman *et al.* is different from the library used in the instantly claimed methods. In the instantly claimed methods, the activity of members of a library containing protease muteins that have N mutations relative to a wildtype scaffold protease or catalytically active portion thereof for a target protein or sustrate sequence therein is assessed. As discussed in the last response, no *a priori* knowledge of the mutations or activity of the protease variants is required. By virtue of practice of the method, protease variants are identified that have increased cleavage activity and/or substrate specificity for a target protein involved in a disease or pathology.

In contrast, the method of Kolterman *et al.* **requires** that all protease variants are related to a first substrate and have a known substrate specificity. Waugh *et al.* does not cure this defect, since it does not teach or suggest any protease library.

2) The instant claims include a step whereby protease muteins are identified that have increased cleavage activity and/or substrate specificity for a substrate sequence in a target protein involved in a disease or pathology **relative to the wild-type** protease scaffold. Kolterman *et al.* teaches a method whereby one or more proteases are selected that has specificity **preferably** for the second substrate. ***Hence, the end-point of the method of Kolterman et al. is distinct from the instant claims.*** The purpose of the methods are different. The instant claims are directed to identifying a protease that has increased cleavage activity and/or altered substrate specificity relative to a wild-type protease. In the methods of Kolterman *et al.* the activity of the wild-type protease is irrelevant to practice of the method; In Kolterman, the specificity of a protease for a first substrate and a second substrate are compared to identify those with specificity preferably for the second substrate.

3) Kolterman *et al.* does not teach or suggest any method where protease muteins are

identified that have increased cleavage activity and/or altered substrate specificity for **a substrate sequence in a target protein involved in a disease or pathology, whereby cleavage results in inactivation of the target protein**. In Kolterman *et al.* the substrate sequence can be any sequence, including an intermediate sequence. There is no teaching or suggestion in Kolterman *et al.* of any method for identifying candidate therapeutics based on a method of identifying from a protease library members that, by virtue of altered cleavage activity and/or substrate specificity for a target protein in a disease or pathology, cleave the target protein thereby inactivating the target protein Waugh *et al.* does not cure this defect. Notwithstanding this, neither Kolterman *et al.* or Waugh *et al.*, singly or in combination, teach or suggest the specific recited target proteins required by all claims for practice of the method.

4) Neither Kolterman *et al.* or Waugh *et al.*, singly nor in any combination, teaches or suggests an iterative method as claimed in independent claim 53 (see also dependent claim 16).

**Analysis**

***Teachings of Kolterman et al. and Waugh et al. and Differences between the Teachings and Instant Claims***

1. Kolterman *et al.* (US2004/0072276)

Kolterman *et al.* is directed to a method for generating a sequence-specific protease that recognizes and cleaves a user-definable amino acid sequence with high specificity. The method involves contacting a population of protease variants specific for a first substrate with a second substrate that contains a specific amino acid sequence resembling the target peptide substrate and selecting one or more protease variants that has specificity preferably for the second substrate. Thus, in the methods of Kolterman *et al.* the population of protease variants exhibit substrate specificity for a first substrate. For example, the first full sentence of column 12 states that "**genes encoding protease variants with know substrate specificities** were PCR amplified...." In addition, claim 1 setting forth the method states that the population of proteases is:

> comprised of variants of one first protease or of variants or chimeras of two or more first proteases, said proteases having a substrate specificity for a particular amino acid sequence of a first peptide substrate.

Hence, the library (i.e. population of protease variants) of Kolterman *et al.* is different from the instant library used in the method as claimed. As claimed in the instant application, a library is produced where each member of the library has N mutations relative to a wild-

type mammalian protease scaffold or catalytically active portion thereof. There is **no requirement** in the instant claims that members of the library have a substrate specificity for a first substrate sequence, nor that the members of the library have to be active. As discussed in the last response, by virtue of practice of the method, only those proteases that are active and that have increased cleavage activity and/or altered specificity for a substrate sequence in a target protein are identified.

Kolterman *et al.* further teaches that the purpose of its method is to "allow the selection of a protease with an increased activity on the desired peptide substrates whereby the activity on the original peptide substrate decreases" (see e.g. at paragraph [0013]. Thus, the method of Kolterman *et al.* screens for proteases to identify a protease mutein that has **preferable specificity for a second substrate compared to a first substrate**. For example, at paragraph [0084] Kolterman *et al.* states:

> It is an essential part of the invention that proteases with the target specificity are generated by selecting protease variants under conditions that allow identification of proteases that recognize and cleave **the target sequence preferably**. [Emphasis added]

In addition, claim 1 of Kolterman *et al* states that the final step of the method includes:

> (c) selecting one or more protease variants from the population of proteases provided in step (a) having specificity for said amino acid sequence of the second substrate provided in step (b) under conditions that allow identification of proteases that recognize and hydrolyze **preferably** said specific one amino acid sequence within the second substrate. [Emphasis added]

In order to identify a substrate that preferably is cleaved compared to a first substrate, Kolterman *et al.* provides various approaches that can be used for comparison of activity of the proteases for various substrates. These include a competitor approach where a competitor substrate that resembles the target substrate is added in high excess. Kolterman *et al.* teaches the approach can identify proteases that are qualitatively specific, but have low quantitative specificity. Kolterman *et al.* also teaches an affinity approach where low target substrate concentration is used; a comparison approach where two or more substrates are contacted with the population of proteases; and an approach using intermediate substrates where substrates are used having intermediate characteristics and are evolved gradually towards the target substrate. For example, at paragraph [0024], Kolterman *et al.* states that these various methods can be used to screen for catalytic activities against different substrates:

> The identification and selection of proteases that have evolved toward the target specificity is done by screening for catalytic activities on **different peptide substrates**, either by screening for increased affinity or by using two substrates in comparison, or by using unspecific peptides as competitors, or by

using intermediate peptide substrates. [Emphasis added]

Kolterman *et al.* further teaches that these various approaches can be combined. In one example described in Figure 3, the competitor approach and affinity approach are used to identify an evolved protease by comparison of activity toward a first substrate and target substrate, see e.g. at paragraph [0116], which states:

> These proteases are selected by screening with an excess of competing substrates (competitor approach) or by screening for higher substrate affinity (affinity approach). In general, the evolved protease can be identified by the **comparison of the catalytic activity towards offered substrates including the first substrates and the target substrates.** [Emphasis added]

Hence, the method of Kolterman *et al.* requires comparison of specificity of a protease for different substrates, generally at least a first substrate and a second target substrate, to identify a protease that preferably cleaves the second substrate.

In contrast, in the instant claims, the method includes a final step of measuring the cleavage activity and/or substrate specificity of members of the library for **a single substrate** sequence or a target protein involved in a disease or pathology, and then identifying those members that have a cleavage activity that is increased or substrate specificity that is altered towards the substrate sequence **relative to the wild-type protease scaffold**. Thus, in the instant method, identification of proteases requires no comparison between two different substrates as taught in Kolterman *et al.* ***Thus, it respectfully is submitted that the end-point of the method includes a different step. As a result, the method of Kolterman et al. achieves a different purpose from the instant method. In the instant method, protease specificity is evolved compared to the wild-type protease. In Kolterman et al., the protease specificity is evolved so that the protease is more selective for one substrate over another substrate.***

Further, in the method of Kolterman *et al.* the substrate can be any substrate. For example, Kolterman *et al.* states that the term "substrate" or "peptide substrate" means "any peptide, oligopeptide, or protein molecule of any amino acid composition, sequence or length, that contains a peptide bond that can be hydrolyzed catalytically be a protease" (see e.g. at paragraph 0039). Thus, Kolterman *et al.* is not directed to a method of generating and screening proteases that cleave proteins known to be involved in disease, but rather is a method to evolve a protease for any substrate. There is no teaching or suggestion in Kolterman *et al.* that the target substrates are substrates involved in a disease. The only exemplary substrate provided by Kolterman *et al.* is the peptide substrate CPGR/VVGG,

which is a substrate of tissue plasminogen activator. This substrate sequence is included in plasminogen, and when cleaved by tPA, **activates** plasminogen to plasmin. In contrast, the instant claims require that the target substrate is a substrate sequence in a target protein involved in a disease or pathology whose inactivation ameliorates the symptoms of a disease or pathology, whereby cleavage **inactivates** the target protein (see e.g. at paragraph 50 of the instant specification). Kolterman *et al.* provides no teaching or suggestion of a method to identify protease muteins that can serve as candidate therapeutics by virtue of increased cleavage activity and/or substrate specificity for a target protein involved in a disease or pathology, where cleavage of the target protein inactivates the target protein. No such target proteins are taught or suggested in Kolterman *et al.*

In addition, there is no teaching or suggestion in Kolterman *et al.* of a method of identifying a protease mutein from among a library of protease muteins that has increased cleavage activity and/or substrate specificity for any of the specifically claimed target proteins relative to a wild-type protease scaffold. Further, Kolterman *et al.* does not teach or suggest a method where mutations of identified proteases are combined to create further mutein proteases. The instant claims are directed to a method of identifying protease muteins with increased cleavage activity and/or altered substrate specificity for specific recited target proteins involved in a disease or pathology. For example, instant claim 1 and 59 recite that the target protein is a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor or a signaling protein that regulates apoptosis. Other dependent claims recite specific target proteins including, but not limited to, a VEGF, VEFG-R, caspase-3 (claim 12) or target proteins recited in claim 62 or claim 63. Kolterman *et al.* does not teach or suggest any of the recited target substrates.

Finally, as acknowledged by the Examiner, Kolterman *et al.* does not teach or suggest a method where the scaffold includes many of the recited scaffolds as claimed. For example, the scaffolds MT-SP1, a granzyme, urinary plasminogen activator and others are not taught or suggested in Kolterman *et al.*

2. Waugh *et al.* (Nature Structure Biology 7: 762-765 (2000)

As discussed in the last response, Waugh *et al.* fails to cure the deficiencies of Kolterman *et al.* Waugh *et al.* was cited by the Examiner because it is directed to the description of granzyme B. Waugh *et al.* teaches that granzymes are involved in inducing apoptosis by acting on downstream substrates such as caspases by **activation** cleavage. Waugh *et al.* teaches that cleavage of caspases by granzymes result in activation of the

substrate. Waugh *et al.* further describes the structure of granzyme B, and elucidation of the molecular determinants of specificity therefrom. Waugh *et al.* teaches the residues in granzyme B that play a role in determining substrate specificity as deduced from a three dimensional structure of granzyme B in complex with a macromolecular inhibitor, ecotin.

Waugh *et al.* does not teach or suggest any of the instantly claimed methods. Waugh *et al.* does not teach or suggest the other elements of the method as claimed for which Kolterman *et al.* is deficient. There is no teaching or suggestion in Waugh *et al.* of a method of evolving granzyme B to identify candidate therapeutics that cleave a substrate sequence in a target protein, thereby inactivating the target protein. Waugh *et al.* teaches that granzyme cleaves substrates (e.g. caspases) in order to activate them. There is no teaching or suggestion of any method in which granzymes, including granzyme B or any other protease, is modified to inactivate a substrate involved in a disease or pathology, thereby serving as a treatment of the pathology.

There is no teaching or suggestion in Waugh *et al.* of a method for producing and indentifying a mutein granzyme B protease that has increased cleavage activity and/or substrate specificity for any target protein involve in a disease or disorder, including any target protein recited in the claims, compared to wild-type granzyme B. Hence, Waugh *et al.* fails to cure the deficiencies in the teachings of Kolterman *et al.* Waugh *et al.*, in fact, is not relevant to the instant claims.

***The combination of teachings of the cited references does not result in the instantly claimed methods.***

The teachings of the references, singly or in combination, fails to teach of suggest all (or even any) elements as claimed. Each of independent claims 1, 53, 59 and 63 is directed to a method including steps of 1) producing a library of protease muteins each having N mutations relative to a wild-type protease; 2) measuring the cleavage activity and/or substrate specificity of at least two members in the library for a substrate sequence in particular recited target proteins involved in a disease or pathology, whereby cleavage of the substrate sequence in the target protein inactivates the target protein; and 3) identifying those protease muteins in the library that have an increased cleavage activity and/or altered substrate specificity relative to a wild-type scaffold protease, thereby identifying a protease mutein that is a candidate therapeutic for treatment of the pathology. Neither Kolterman *et al.* or Waugh *et al.*, singly or in any combination, teaches or suggests any of the above steps.

*a) Kolterman et al. or Waugh et al., singly or in combination, does not teach or suggest producing a protease mutein library as claimed*

For example, the instant claims are directed to a method including s step of producing a library, whereby the library includes **any** mutein protease that has N mutations relative to a wildtype scaffold protease or catalytically active portion thereof. In Kolterman *et al.*, the library, while being a library of protease variants of one or more proteases, is a library where every member of the library has the same substrate specificity for a first substrate. **Thus, the library used in the method of Kolterman *et al.* is different from the library used in the instantly claimed method.** Furthermore, Waugh *et al.* does not cure this defect, since Waugh *et al.* does not teach or suggest any protease library. Waugh *et al.* provides no teaching or suggestion of any method in which any muteins in a library are identified, or even in any method of generating or testing mutant proteases.

*b) Kolterman et al. or Waugh et al., singly or in combination, does not teach or suggest a method that includes contacting members of a library of protease muteins with a polypeptide containing substrate sequence from a target or with a target, where the target is one whose inactivation ameliorates the symptoms of a disease or pathology.*

*c) Kolterman et al. or Waugh et al., singly or in combination, does not teach or suggest a method including a step of measuring the cleavage activity and/or substrate specificity of protease muteins for a substrate sequence in a target protein involved in a disease or pathology as claimed, and identifying protease muteins as candidate therapeutics therefrom*

The instant claims include a step where members of the library are tested for cleavage activity and/or substrate specificity for a substrate sequence in a **target protein involved in a disease or pathology, whereby cleavage of the substrate sequence in the target protein <u>inactivates</u> an activity of target protein involved in a disease or pathology**. The method of Kolterman *et al.* is directed to assessing cleavage activity for <u>any second substrate</u> so long as it is different from a first substrate. The second substrate can be anything. The only substrate exemplified by Kolterman *et al.* is the peptide substrate CPGRVVGG (and intermediates thereof), which is a substrate sequence in plasminogen required for activation of plasminogen to plasmin. There is no teaching or suggestion in Kolterman *et al.* of any substrate sequence that, when cleaved, results in inactivation of an activity in the target protein involved with a disease or pathology. Waugh *et al.* does not cure this defect. Waugh *et al.* teaches that granzymes act on caspase substrates by activation cleavage. There is no teaching or suggestion in either Waugh *et al.* or Kolterman *et al.* of any method of identifying a candidate therapeutic that inactivates an activity of a target protein involved in a disease or disorder.

*d) Kolterman et al. or Waugh et al., singly or in combination, does not teach or suggest any of the recited target proteins used in the methods as claimed*

The method as instantly claimed is directed to a method of identifying protease muteins that inactivate an activity of particular classes of target proteins or specific target proteins. Neither Kolterman *et al.* or Waugh *et al* teaches or suggests any of such target proteins using in the methods as claimed. Specifically, there is no teaching or suggestion in Kolterman *et al.* of any method that targts the specific recited classes of target proteins or the specific recited target proteins in independent claims 1, 59, 63 and/or 66. For example, Kolterman *et al.* does not teach or suggest a method in which the target substrate is a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis, nor any specific proteins recited, including caspase, in independent claims 1, 59 and 63 or dependent claim 62 or 66. There is no teaching or suggestion in Kolterman *et al.* of a method of identifying a protease mutein from among a library of protease mutein that has increased cleavage activity and/or substrate specificity for a substrate sequence in any of the recited classes of target proteins or any of the specific target proteins claimed, where cleavage of a substrate sequence inactivates an activity of the target protein. Waugh *et al.* does not cure this defect. Although Waugh *et al.* teaches that caspase is a natural substrate of granzyme, Waugh *et al.* teaches that granzymes are required to cleave caspases in order to activate the caspases. There is no teaching or suggestion in Waugh *et al.* of any method to modify granzyme, or any other protease, to have altered substrate specificity relative to wild-type granzyme B to inactivate caspase as a treatment for a disease or pathology.

*e) Kolterman et al. or Waugh et al., singly or in combination, do not teach or suggest a method were protease muteins are identified that have increased cleavage activity and/or substrate specificity relative to a wild-type protease as instantly claimed.*

In addition, neither Kolterman *et al.* or Waugh *et al.* teaches or suggests a method that results in identification of proteases that have increased cleavage activity and/or substrate specificity **relative to a wild-type protease**. The instant claims are directed to a method including a step where protease muteins are identified that have increased cleavage activity and/or substrate specificity for a substrate sequence in a target protein involved in a disease or pathology **relative to the wild-type** protease scaffold. The method of Kolterman *et al.* includes a step with an entirely different end-point. Kolterman *et al.* teaches a method whereby one or more proteases are selected that has specificity preferably for the second substrate. Hence, the point of the method of Kolterman *et al.* is to identify a protease mutein

that has preferential specificity for a second substrate over a first substrate. Thus, in the method of Kolterman *et al.*, the protease muteins that are identified do not necessarily have increased cleavage activity and/or specificity compared to the wildtype scaffold protease. The instant claims require that the protease muteins have an increased specificity and/or activity compared to a wild-type protease. In particular, the claims require that the specificity is increased by at least 10-fold (claim 13), 100-fold (claim 14) or 1000-fold (claim 15). Waugh *et al.* does not cure this defect, since Waugh *et al.* does not teach or suggest any method involving the assessment of specificity or cleavage activity of any protease.

*f) Kolterman et al. or Waugh et al., singly or in combination, further does not teach or suggest other elements as claimed*

Notwithstanding that Kolterman *et al.* or Waugh *et al.*, singly or in combination, do not teach or suggest any of the steps of the method as claimed, they also do not teach or suggest many of the other elements as claimed, for example, as recited in rejected dependent claims. For example, neither Kolterman *et al.* or Waugh *et al.* teaches a method that includes a step of combining mutations from identified mutein proteases to generate a third protease, and then measuring the cleavage activity and/or substrate specificity thereof to determine if it is increased or altered compared to the first or second mutein protease (see e.g. independent claim 53 and dependent claim 16) . Further, there is no teaching or suggestion of many of the specific recited proteases as claimed. There also is no teaching or suggestion in either reference of a method of identifying a protease mutein that cleaves a target protein in a disease or pathology where the disease or pathology is any set forth in claim 9. There is no teaching or suggestion in either Kolterman *et al.* of Waugh *et al.* of identifying protease muteins as candidate therapeutics to inactivate any target protein, in particular any target protein involved in the recited diseases or pathologies.

***Conclusion***

Since none of the steps of the method are taught or suggested by Kolterman *et al.* or Waugh *et al.*, alone or in combination, the combination of teachings cannot result in the instantly claimed method. For example, the source of mutein protease (i.e. library) is different in the instantly claimed method from the teachings of Kolterman *et al.* (or Waugh *et al.* who doesn't even teach a library). In addition, the substrate sequence is different, since in Kolterman *et al.* the substrate sequence can be anything. There is no teaching or suggestion in Kolterman *et al.* that the substrate sequence is a sequence in a target protein, whereby cleavage of the substrate sequences results in inactivation of an activity of the target protein.

Waugh *et al.* teaches no substrate sequences, and therefore, does not cure this defect. Further, the end-point of the method of Kolterman *et al.* and the instantly claimed method is different such that the resulting mutein protease are identified based on different properties. Kolterman *et al.* teaches a method where those proteases that have preferential cleavage activity for a second substrate over a first substrate are identified. The instant claims are directed to a method where protease muteins are identified based on activity relative to a wild-type protease. Even assuming the other steps of the method were the same, different protease muteins would be identified based on the differing end-points of the method. Finally, neither Kolterman *et al.* or Waugh *et al.* teaches or suggests any of the particular recited target proteins as claimed. Hence, the combination of teachings of Kolterman *et al.* and Waugh *et al.* cannot result in the instantly claimed method. Since a *prima facie* case of obviousness requires that the references, singly or in combination, must teach all elements as claimed, the Examiner has failed to set forth a case of *prima facie* obviousness

***Rebuttal to Examiner's Comment***

*1) The Examiner states that, while Kolterman et al. does not describe granzyme B in their method, one of ordinary skill in the art would have been motivated to use granzyme for the advantage taught by Waugh that granzyme is involved in rapid cell death of a tumor cell.*

It respectfully is submitted that although Kolterman *et al.* does not teach all elements as claimed for the reasons discussed above, even if it did, there would be no motivation to have selected granzyme as a scaffold. The instant claims are directed to the identification of mutein proteases, in particular mutein proteases that inactivate a target protein involved in a disease or pathology. Such protease muteins are identified as candidate therapeutics. Thus, the point of the method is to identify protease muteins. In contrast, the Examiner seems to be implying that granzyme is a good protease to use in the method because wild-type granzyme is involved itself in rapid death of a tumor cell. The instant claims, however, are directed to modifying the substrate specificity and or cleavage activity of granzyme B. Thus, one of ordinary skill in the art would not have been motivated to modify granzyme B because it already acts to kill tumor cells.

Notwithstanding this, Waugh *et al.* states that granzyme B is involved in contributing to rapid cell death of a tumor cell by the activating cleavage of caspases. The instant claims are directed to identification of protease muteins that inactivate a target protein. As discussed above, there is no teaching or suggestion in Waugh *et al.* or Kolterman *et al.*, singly or in any combination, of any method of identifying protease muteins that inactivate a target protein involved in a disease or pathology.

*2) The Examiner states that Applicant's arguments in the last response were not commensurate with the elected species of the target protein, i.e. caspase.*

It respectfully is submitted that Applicant's arguments in the last response were made to address the differences in the teachings of Kolterman *et al.* and Waugh *et al.*, singly and in combination, to address a *prima facie* case of obviousness for the claims as pending, including with respect to the elected species and the encompassing embodiments. For a *prima facie* case of obviousness, the combination of the references must teach or suggest all elements as claimed. Since the instant claims are directed to a method, all elements of the method as claimed were addressed and differences so stated. The specific substrate sequence in a target protein (i.e. caspase) used in practice of the method is but one element of the method. But, among the differences stated in Applicant's arguments in the last response were that neither Kolterman *et al.* or Waugh *et al.* teaches or suggests a method of identifying a protease mutein that has altered cleavage activity and/or substrate specificity for **any** target protein involved in a disease or pathology. This necessarily means that Kolterman *et al.* and/or Waugh *et al.* do not teach or suggest a method of identifying a protease mutein that has altered cleavage activity and/or substrate specificity for caspase. Thus, it respectfully is submitted that Applicant's arguments addressed the elected species.

Further, election of species is a search tool. Applicant is not prohibited from providing arguments when a claim is generic to the elected species. In this instance, it respectfully is submitted that neither Kolterman *et al.* nor Waugh *et al.*, singly or in combination, teaches or suggests such a method as claimed for the reasons discussed above. Accordingly, it respectfully is submitted that Kolterman *et al.* or Waugh *et al.*, singly or in any combination, does not teach or suggest a method as claimed where the protease scaffold is a granzyme B and the substrate sequence in a target protein is a caspase. Thus, Applicant has demonstrated patentability of the generic claims as well as the encompassed species.

* * *

Entry of this amendment and examination of the application are respectfully requested.

Respectfully submitted,

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